PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 April 2001 (09.04.01)	
International application No. PCT/EP00/07874	**Applicant's or agent's file reference** K1596-PCT
International filing date (day/month/year) 08 August 2000 (08.08.00)	**Priority date** (day/month/year) 10 August 1999 (10.08.99)
Applicant DECKMYN, Hans et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

28 February 2001 (28.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Zakaria EL KHODARY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
K1596-PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 07874	08/08/2000	10/08/1999
Applicant		
K.U.LEUVEN RESEARCH & DEVELOPMENT		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 7

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]T/EP 00/07874

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/20 C07K16/28 A61K39/395 C12N15/13 C12Q1/68
A61P7/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EMBASE, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	F. PARETI ET AL.: "Interaction of porcine von Willebrand factor with the platelet glycoproteins Ib and IIb/IIIa complex." BRITISH JOURNAL OF HAEMATOLOGY, vol. 82, no. 1, September 1992 (1992-09), pages 81-86, XP000914679 Oxford, GB abstract --- -/--	2-8,10, 11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2001	21/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	B. BECKER ET AL.: "Effects of an antiplatelet glycoprotein Ib antibody on hemostatic function in the guinea pig." BLOOD, vol. 74, no. 2, 1 August 1989 (1989-08-01), pages 690-694, XP000914660 New York, NY, USA abstract * discussion *	2-8,10, 11,14, 16,18,19
X	US 5 336 667 A (KIRBY ET AL.) 9 August 1994 (1994-08-09) the whole document	3,14,16, 18,19
A	J. WARD ET AL.: "Epitope and functional characterization of the CD42 (GPIB/IX) MAB panel." In: Leucocyte typing V: White cell differentiation antigens. vol. 2, no. 2, 1995, pages 1336-1337. XP002110444 the whole document	1-25
A	US 5 486 361 A (U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES) 23 January 1996 (1996-01-23) the whole document	1-25
P,X	N. CAUWENBERGHS ET AL.: "Antithrombotic effect of platelet glycoprotein Ib-blocking monoclonal antibody Fab fragments in nonhuman primates." ARTERIOSCLEROSIS, THROMBOSIS AND VASCULAR BIOLOGY, vol. 20, no. 5, May 2000 (2000-05), pages 1347-1353, XP000914634 Dallas, TX, USA the whole document	1-25
P,X	WO 00 26667 A (J. MILLER) 11 May 2000 (2000-05-11) page 42, line 1 - line 11 claims	2-16, 18-21

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
T/EP 00/07874

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5336667 A	09-08-1994	AU 3232393 A	28-06-1993
		MX 9206960 A	01-12-1993
		WO 9311151 A	10-06-1993
US 5486361 A	23-01-1996	NONE	
WO 0026667 A	11-05-2000	AU 1458500 A	22-05-2000
		EP 1051620 A	15-11-2000

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
K1596-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/07874	08/08/2000	10/08/1999
International Patent Classification (IPC) or national classification and IPC C07K16/28		
Applicant K.U.LEUVEN RESEARCH & DEVELOPMENT		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/03/2001	16.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mueller, F Telephone No. +49 89 2399 7722

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/07874

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-4,7-30	as originally filed			
5,6	as received on	11/10/2001	with letter of	11/10/2001

Claims, No.:

1-43	as received on	11/10/2001	with letter of	11/10/2001

Drawings, sheets:

1/11-11/11	as originally filed

Sequence listing part of the description, pages:

4, filed with the letter of 11.10.2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☒ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☐ claims Nos. .

because:

   ☒ the said international application, or the said claims Nos. 30-37 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear

that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-43
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-43
Industrial applicability (IA)	Yes:	Claims	1-43 (30-37?)
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of the report

Sequence listings filed, 4 pages, Seq ids 1-4, with the letter of 06.12.2000 and 11.10.2001, are filed after the filing date of the application and do not form part of the description and will not be annexed to this communication/report (Rule 13ter.(f) PCT).

Re Item II

Priority

The subject-matter of claims 1-38 is entitled to the claimed priority date (10.08.1999). The subject-matter of claim 39 is entitled to claimed priority date of 02.02.2000. The subject-matter of claims 40-43 is not entitled to any claimed priority dates, therefore the relevant date for this subject-matter is the date of filing (08.08.2000).
Therefore the cited P-document (Cauwenberghs et al., published 05.2000) and D4 (published 11.05.2000) of the International Search Report is relevant prior art for subject-matter of claim 40-43 in respect to inventive step within the meaning of Article 33 (3) PCT.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 30-37 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Articl 35(2) with regard to nov lty, inventiv step or industrial applicability; citations and explanations supporting such statement

The arguments presented by the applicant with the letter of 11.10.2001 are taken into account.

1. Reference is made to the following documents:

   D1: F. PARETI ET AL.: BRITISH JOURNAL OF HAEMATOLOGY, vol. 82, no. 1, September 1992 (1992-09), pages 81-86,
   D2: B. BECKER ET AL.: BLOOD, vol. 74, no. 2, 1 August 1989 (1989-08-01), pages 690-694,
   D3: US-A-5 336 667
   D4: WO-A1-002667
   D5: N. CAUWENBERGHS ET AL.: ARTERIOSCLEROSIS, THROMBOSIS AND VASCULAR BIOLOGY, vol. 20, no. 5, May 2000 (2000-05), pages 1347-1353

2. The subject-matter of claim 1 is novel (Article 33 (2) PCT).

2.1 The subject-matter of claim 1 is not inventive (Article 33 (3) PCT).
D1 describes a monoclonal antibody, LJCP1, which is able to bind GPIb and therewith inhibits the binding of von Willebrand factor (see abstract and p.82, 2.col. last par.-p.83, 2.col. 1.par.; p.85,1.col., 1.par.). D1 therefore is considered to provide a method for inhibiting the interaction of von Willebrand factor with platelets which interact with the formation of thrombocytopenia (p. 81, 1.col., 1.par.).

D2 discloses the murine monoclonal antibody, PG-1, which recognizes GPIb in guinea pig platelets and therefore also inhibits the von Willebrand factor dependent platelets agglutination (see abstract and p.690, 1.col., 1.par.). The action of full PG-1 antibody and fragments thereof (F(ab)2) were tested on prolongation of the template bleeding time (see p. 693, 1.col., 1. par.), which shows no significant prolongation of the template bleeding time by the application of F(ab')2. Furthermore D2 discusses the potential role of the PG-1 antibody in antithrombotic treatments (p. 693, 1. col., 1. par. and 2. col., last par.).

D1 and D2 are regarded as close prior art for the subject-matter of claim 1.
The subject-matter of claim 1 differs to D1 or D2 by providing a cell line, LMBP 5108CB, which is producing an antibody having the same functional features as the antibodies described in D1 or D2, namely the binding to GPIb and therewith inhibiting the binding of von Willebrand factor to GPIb.
The problem to be solved by the present application (Claim 1) may therefore be regarded as providing a different antibody.
The solution is given in the present application by providing the monoclonal antibody 6B4 which is produced by the cell line LMBP5108CB.
The production of monoclonal antibodies by hybridoma techniques is considered to be a standard procedure in this technical field. In addition also if D1 not provides experimental data for the in vivo use of the LJCP1 antibody, D1 would prompt the person skilled in the art to use such antibodies or fragments thereof, which have the same technical features as the LJCP1 antibodies (namely the binding to GPIb), also in vivo. As no other special technical and functional features for the antibody 6B4, in comparison to the antibodies which are already described in the prior art (see D1 or D2), can be detected, the provision of antibody 6B4 is considered as an alternative solution to an already solved problem. The requirements for inventive step for the antibody as well for the cell line producing it are therefore not fulfilled (Article 33 (3) PCT).

2.2 Thereon dependent claims 2-6 are considered not to introduce additional technical features which in the light of the prior art (D1 and D2) seems to be special. Thus an inventive step for the subject-matter of claims 2-6 can not be acknowledged.

3. The subject-matter of claim 7 is not inventive (Article 33 (3) PCT).
As it has been already discussed under point 2.1 (see above) D1 and D2 provide antibodies and fragments thereof which are able to bind to GPIb and therewith inhibit the interaction of the von Willenbrand factor with GPIb. Also the provision of Fab fragments for in vivo application seems to be standard procedure in the technical field and therefore can not be acknowledged as inventive.

The same hold true for the subject-matter of claim 8.

3.2 The subject-matter of thereon dependent claims 9-17 and claims 18-37 seems not to introduce additional subject-matter which can be acknowledged as inventive with respect to D1 and D2. D2 also describes the in vivo use of a monoclonal antibody, PG-1, in a concentration of 1.3mg/kg, see abstract, and therefore already describes a concentration which falls within the range of the claimed subject-matter of claim 31. Thus the requirements of Article 33(3) PCT for claims 9-17 and 18-37 are not fulfilled.

4. The subject-matter of claims 38,39 and 40-43 is novel (Article 33 (2) PCT).
The subject-matter of claims 20,21 and 22-25 lacks inventive step (Article 33 (3) PCT).

Following the reasoning that the claimed antibody and fragments thereof lacks inventive step (see 2.1) also the provision of amino acid sequences, nucleic acid sequences and DNA probes therefore is considered at present as a routine skill in particular also in view of D4 and D5, the subject-matter of claims 38,39 and 40-43 therefore also does not fulfil the requirements of inventiveness (Article 33 (3) PCT).

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO-A1-0026667	11.05.2000	29.10.1999	30.10.1998

The intermediate document discloses antibody fragments capable of inhibiting von Willebrand factor dependent aggregation of platelets by binding to GpIb and therefore are useful as anti-thrombotic agents (see page 33, lines 7-24).
This document therefore could play a role in the national or regional phase (EPO

(Article 54(3) EPC) in respect to novelty of claims 7,8,18,20,30,32,38 and 39 and novelty/inventive step to subject-matter not entitled to the claimed priority (claims 40-43).

Re Item VIII

Certain observations on the international application

1. The expression "homologue" in claims 7-13,25-30,32 and 36-38 is not clear (Article 6 PCT).
   The homologues should be defined by technical features e.g. amino acid sequences.

2. The expressions "shear" or "high shear" condition in claims 11 and 12 are not clear (Article 6 PCT) and therefore should be defined by the definition given in the description e.g. on p.18, line 18.

thrombogenesis. They include the use of anti-vWF monoclonal antibodies, GPIb binding snake venom proteins like echicetin and crotalin, aurin tricarboxylic acid that binds to vWF and recombinant vWF fragments like VCL, all of which inhibit vWF-GPIb interaction. All these molecules were anti-thrombotic, particularly in studies where a thrombus was formed under high shear conditions. U.S.Patent 5,486,361 discloses a monoclonal antibody 4H12 which specifically binds to the α chain of GPIb and, by means of this interaction, totally inhibits the binding of thrombin to normal human platelets. In addition, it inhibits more than 90% of thrombin-induced von Willebrand factor or fibrinogen binding to platelets. Further, 4H12 does not inhibit ristocetin- or botrocetin-induced binding of von Willebrand factor to platelet cells, which indicates that this antibody does not prevent von Willebrand factor binding to GPIb. A number of potent inhibitory anti-GPIb antibodies, such as LJIb1 disclosed by F.Pareti et al. in *British Journal of Haematology* (1992) 82, 81-86, have been produced and were extensively tested with respect to their *in vitro* effect under both static (platelet agglutination, vWF-binding) and flow conditions. However for none of these anti-human GPIb antibodies an *in vivo* anti-thrombotic effect could be demonstrated. *In vivo* data obtained by B.Becker and J.L.Miller (*Blood* (1989)2:680-694) describe the effect of injecting guinea pigs with intact antibody or $F(ab')_2$ fragments of PG1, a monoclonal anti-guinea pig GPIb antibody. After intraperitoneal injection of the intact antibody, a hemorrhagic state was produced with a significant lengthening of the bleeding time and drop of the platelet count to 50% of its baseline value. Injection of 0.63 to 2.5 mg/kg of the $F(ab')_2$ fragments did not decrease the platelet count more than 21%, and bleeding times never increased by more than one minute over baseline values. However, in this particular study the antithrombotic effect of the $F(ab')_2$ fragments was not further investigated by e.g. testing the fragments in an animal thrombosis model. In a follow-up study J.L.Miller et al., *Arterioscler.Thromb.* (1991) 11:1231-6 disclosed that the $F(ab')_2$ fragments of PG1 in guinea pigs using these could effectively reduce thrombus formation on a laser-induced injury. Unfortunately, this antibody does not cross react with human plat lets and therefore it has no further clinical relevance for human therapy.

Part of this rather surprising lack of *in vivo* studies is due to the low cross reactivity of the anti-human GPIb monoclonal antibodies with platelets from commonly used laboratory animals. This predisposes to the use of non-human primates as experimental animals. However, even then attempts to perform *in vivo* studies are hampered because injection of the anti-GPIb monoclonal antibodies, as well as the snake venom protein echicetin that reacts with GPIb, invariably causes severe thrombocytopenia, as taught by US-A-5,336,667. WO-A-002667 further discloses monoclonal antibodies F_{ab} fragments but does not discuss thrombocytopenia and does not mention *in vivo* tests.

One persistent concern with all available thrombolytic and anti-thrombotic agents, including aspirin, is to induce a risk of overdose and therefore of excessive and life-threatening bleeding. Therefore a first goal of the present invention is to provide a thrombus formation protective means by providing a platelet adhesion inhibitor that does not induce a risk of bleeding. A second goal of the present invention is to provide a thrombus formation protective means by providing an inhibitor of platelet adhesion without incurring the risk of thrombocytopenia. A third goal of the present invention is the targetting of platelet adhesion, activation and aggregation under high shear conditions, which is of particular importance in the setting of highly stenotic atherosclerotic lesions. The specific targetting of highly stenotic areas in the circulation should make GPIb inhibition particularly suitable for treating unstable angina and in the chronic prevention of arterial occlusion. Unlike with GPIIb/IIIa inhibition, platelet aggregation as well as hemostasis is not expected to be inhibited in low shear vessels, i.e. in veins and normal arteries. Bleeding complications from these vessels by inhibition of GPIb may therefore be expected to be better reduced than with GPIIb/IIIa inhibition.

SUMMARY OF THE INVENTION

The essence of this invention is that by using a ligand such as a monovalent Fab fragment of a certain inhibitory human GPIb antibody, a marked prevention of platelet d pendent thrombus formation targetted to high shear flow vessels and without incurring thrombocytopenia can be obtained. Moreover, this is so far the only anti-human GPIb monoclonal antibody for

CLAIMS

1. A cell line deposit d with the Belgian Coordinated Collections of Microorganisms, under accession number LMBP 5108CB, being able to produce a monoclonal antibody comprising a F_{ab} fragment which binds *in vivo* to human platelet glycoprotein GPIb.

2. A cell line producing a monoclonal antibody having a reactivity identical to that of a monoclonal antibody obtained from the cell line of claim 1.

3. A cell line according to claim 1 or claim 2, wherein the monoclonal antibody F_{ab} fragment further prevents the binding of von Willebrand factor to human platelet glycoprotein GPIb.

4. A cell line according to any of claims 1 to 3, wherein the monoclonal antibody F_{ab} fragment further inhibits platelet adhesion.

5. A cell line according to any of claims 1 to 4, wherein the monoclonal antibody F_{ab} fragment further inhibits platelet activation under high shear conditions.

6. A cell line according to any of claims 1 to 5, wherein the monoclonal antibody F_{ab} fragment further inhibits platelet aggregation under high shear conditions.

7. A F_{ab} fragment, or a homologue having at least 60% amino acid sequence identity therewith, of a monoclonal antibody which binds *in vivo* to human platelet glycoprotein GPIb without incurring thrombocytopenia.

8. A monoclonal antibody F_{ab} fragment or a homologue threreof according to claim 7, which prevents the binding of von Willebrand factor to human platelet glycoprotein GPIb.

9. A monoclonal antibody F_{ab} fragment or a homologue thereof according to claim 7 or claim 8, which does not produce thrombocytopenia when administered to a primate at a dose of up to at least 4 mg/kg by bolus intravenous administration.

10. A monoclonal antibody F_{ab} fragment or a homologue thereof according to any of claims 7 to 9, which further inhibits platelet adhesion.

11. A monoclonal antibody F_{ab} fragment or a homologue thereof according to any of claims 7 to 10, which further inhibits platelet activation under high shear conditions.

12. A monoclonal antibody F_{ab} fragment or a homologue thereof according to any of claims 7 to 11, which further inhibits platelet aggregation under high shear conditions.

13. A monoclonal antibody comprising a F_{ab} fragment or a homologue thereof according to any of claims 7 to 12.

14. A monoclonal antibody according to claim 13, being produced by on purpose immunization in animals.

15. A monoclonal antibody obtainable from the cell line of claim 1.

16. A monoclonal antibody according to claim 15, being the murine monoclonal antibody 6B4.

17. A monoclonal antibody obtainable from a cell line according to any of claims 2 to 6.

18. A humanized monoclonal antibody derivable from the cell line of claim 1 or from a monoclonal antibody according to claim 15 or claim 16.

19. A humanized monoclonal antibody obtainable from a cell line according to

any of claims 2 to 6 or from a monoclonal antibody according to claim 17.

20. A pharmaceutical composition comprising a monoclonal antibody F_{ab} fragment or a homologue thereof according to any of claims 7 to 12 in admixture with a pharmaceutically acceptable carrier.

21. A pharmaceutical composition according to claim 20, further comprising a therapeutically effective amount of a thrombolytic agent.

22. A pharmaceutical composition according to claim 21, wherein the thrombolytic agent is selected from aspirin, heparin, tissue plasminogen activators, streptokinase, reptilase and staphilokinase.

23. A pharmaceutical composition according to any of claims 20 to 22, for the prevention or treatment of a haemostasis disorder.

24. A pharmaceutical composition according to any of claims 20 to 23, for oral, intranasal, subcutaneous, intramuscular, intradermal, intravenous, intraarterial or parenteral administration or for catheterization.

25. A monoclonal antibody F_{ab} fragment or a homologue thereof according to any of claims 7 to 12 for use as a medicament.

26. A monoclonal antibody F_{ab} fragment or a homologue thereof according to claim 25, wherein the medicament is for the prevention or treatment of a haemostasis disorder.

27. A monoclonal antibody F_{ab} fragment or a homologue thereof according to claim 25 or claim 26, for simultaneous or sequential association with at least a thrombolytic agent.

28. A monoclonal antibody F_{ab} fragment or a homologue thereof according to claim 27, wherein the thrombolytic agent is select d from aspirin, heparin, tissue plasminogen activators, streptokinase, reptilase and staphilokinase.

29. A monoclonal antibody F_{ab} fragment or a homologue thereof according to any of claims 25 to 28, for oral, intranasal, subcutaneous, intramuscular, intradermal, intravenous, intraarterial or parenteral administration or for catheterization.

30. A method of treatment and/or prevention of a haemostasis disorder comprising administering to a patient in need thereof a therapeutically effective amount of a monoclonal antibody F_{ab} fragment or a homologue thereof according to any of claims 7 to 12.

31. A method of treatment and/or prevention according to claim 30, wherein the therapeutically effective amount ranges from 80 µg/kg to 4 mg/kg.

32. A method for the treatment and/or prevention of a haemostasis disorder without inducing thrombocytopenia, comprising administering to a patient in need thereof a therapeutically effective amount of a monoclonal antibody F_{ab} fragment or a homologue thereof according to any of claims 7 to 12.

33. A method of treatment and/or prevention according to claim 32, wherein the therapeutically effective amount ranges from 80 µg/kg to 4 mg/kg.

34. A method according to any of claims 30 to 33, comprising further administration of at least a thrombolytic agent.

35. A method according to claim 34, wherein the thrombolytic agent is selected from aspirin, heparin, tissue plasminogen activators, streptokinase, reptilase and staphilokinase.

36. A method according to claim 34 or 35, wherein the thrombolytic agent is administered simultaneously with the monoclonal antibody F_{ab} fragment or a homologue ther of.

37. A method according to claim 34 or 35, wh rein the thrombolytic agent is administered sequentially with the monoclonal antibody F_{ab} fragm nt or a homologue thereof.

38. A polynucleotide encoding for an antigen-binding monoclonal antibody F_{ab} fragment or a homologue thereof according to any of claims 7 to 12.

39. A DNA probe for detecting the polynucleotide sequence of claim 38, comprising a nucleic acid molecule having a sequence complementary to the coding sequence of said polynucleotide.

40. A polynucleotide sequence as shown in SEQ.N°1.

41. A polynucleotide sequence as shown in SEQ.N°2.

42. An amino acid sequence as shown in SEQ.N°3.

43. An amino acid sequence as shown in SEQ.N°4.

thrombogenesis. They include the use of anti-vWF monoclonal antibodies, GPIb binding snake venom proteins like echicetin and crotalin, aurin tricarboxylic acid that binds to vWF and recombinant vWF fragments like VCL, all of which inhibit vWF-GPIb interaction. All these molecules were anti-thrombotic, particularly in studies where a thrombus was formed under high shear conditions. U.S.Patent 5,486,361 discloses a monoclonal antibody 4H12 which specifically binds to the α chain of GPIb and, by means of this interaction, totally inhibits the binding of thrombin to normal human platelets. In addition, it inhibits more than 90% of thrombin-induced von Willebrand factor or fibrinogen binding to platelets. Further, 4H12 does not inhibit ristocetin- or botrocetin-induced binding of von Willebrand factor to platelet cells, which indicates that this antibody does not prevent von Willebrand factor binding to GPIb.

A number of potent inhibitory anti-GPIb antibodies, such as LJIb1 disclosed by F.Pareti et al. in *British Journal of Haematology* (1992) 82, 81-86, have been produced and were extensively tested with respect to their *in vivo* effect under both static (platelet agglutination, vWF-binding) and flow conditions. However for none of these anti-human GPIb antibodies an *in vivo* anti-thrombotic effect could be demonstrated. *In vivo* data obtained by B.Becker and J.L.Miller (*Blood* (1989)2:680-694) describe the effect of injecting guinea pigs with intact antibody or $F(ab')_2$ fragments of PG1, a monoclonal anti-guinea pig GPIb antibody. After intraperitoneal injection of the intact antibody, a hemorrhagic state was produced with a significant lengthening of the bleeding time and drop of the platelet count to 50% of its baseline value. Injection of 0.63 to 2.5 mg/kg of the $F(ab')_2$ fragments did not decrease the platelet count more than 21%, and bleeding times never increased by more than one minute over baseline values. However, in this particular study the antithrombotic effect of the $F(ab')_2$ fragments was not further investigated by e.g. testing the fragments in an animal thrombosis model.

In a follow-up study J.L.Miller et al., *Arterioscler.Thromb.* (1991) 11:1231-6 disclosed that the $F(ab')_2$ fragments of PG1 in guinea pigs using these could effectively reduce thrombus formation on a laser-induced injury.

Unfortunately, this antibody does not cross react with human platelets and therefore it has no further clinical relevance for human therapy.

Part of this rather surprising lack of *in vivo* studies is due to the low cross reactivity of the anti-human GPIb monoclonal antibodies with platelets from commonly used laboratory animals. This predisposes to the use of non-human primates as experimental animals. However, even then attempts to perform *in vivo* studies are hampered because injection of the anti-GPIb monoclonal antibodies, as well as the snake venom protein echicetin that reacts with GPIb, invariably causes severe thrombocytopenia.

One persistent concern with all available thrombolytic and anti-thrombotic agents, including aspirin, is to induce a risk of overdose and therefore of excessive and life-threatening bleeding. Therefore a first goal of the present invention is to provide a thrombus formation protective means by providing a platelet adhesion inhibitor that does not induce a risk of bleeding. A second goal of the present invention is to provide a thrombus formation protective means by providing an inhibitor of platelet adhesion without incurring the risk of thrombocytopenia. A third goal of the present invention is the targetting of platelet adhesion, activation and aggregation under high shear conditions, which is of particular importance in the setting of highly stenotic atherosclerotic lesions. The specific targetting of highly stenotic areas in the circulation should make GPIb inhibition particularly suitable for treating unstable angina and in the chronic prevention of arterial occlusion. Unlike with GPIIb/IIIa inhibition, platelet aggregation as well as hemostasis is not expected to be inhibited in low shear vessels, i.e. in veins and normal arteries. Bleeding complications from these vessels by inhibition of GPIb may therefore be expected to be better reduced than with GPIIb/IIIa inhibition.

SUMMARY OF THE INVENTION

The essence of this invention is that by using a ligand such as a monovalent Fab fragment of a certain inhibitory human GPIb antibody, a marked prevention of platelet dependent thrombus formation targetted to high shear flow vessels and without incurring thrombocytopenia can be obtained. Moreover, this is so far the only anti-human GPIb monoclonal antibody for

CLAIMS

1. Cell line deposited with the Belgian Coordinated Collections of Micro -organisms, under accession number LMBP 5108CB.

2. A cell line producing monoclonal antibodies having a reactivity substantially identical to that of the monoclonal antibodies obtained from the cell line of claim 1.

3. A ligand which binds to the human platelet glycoprotein GPIb and prevents the binding of von Willebrand factor to said human GPIb.

4. A ligand according to claim 3, which does not produce thrombocytopenia when administered to a primate at a dose of up to at least 4 mg/kg by bolus intravenous administration.

5. A ligand derived from a monoclonal antibody obtainable from the cell lines of claim 1 or claim 2.

6. A ligand according to claim 5, which binds to the human platelet glycoprotein GPIb.

7. A ligand according to claim 5 or claim 6, which prevents the binding of von Willebrand factor to the human platelet glycoprotein GPIb.

8. A ligand according to any of claims 5 to 7, which does not produce thrombocytopenia when administered to a primate at a dose of up to at least 4 mg/kg by bolus intravenous administration.

9. A ligand according to any of claims 5 to 8, being a Fab fragment of the said monoclonal antibody.

10. A ligand according to any of claims 5 to 9, being able to recognize an

epitope located on human platelet glycoprotein GPIb.

11. A ligand according to any of claims 3 to 9 and being derived from a monoclonal antibody produced by on purpose immunization in animals.

12. A humanized or hybridized monoclonal antibody derivable from the monoclonal antibody of claim 11 or derivable from the cell lines of claims 1 or 2.

13. An antigen-binding Fab fragment or a homolog or derivative of a monoclonal antibody according to claims 11 or 12 or derived from the cell lines of claims 1 or 2.

14. A pharmaceutical composition, comprising a ligand according to any of claims 3 to 11, a humanized or hybridized monoclonal antibody according to claim 12 or an antigen-binding Fab fragment according to claim 13, in admixture with a pharmaceutically acceptable carrier.

15. A pharmaceutical composition according to claim 14, further comprising a thrombolytic agent in a form either for simultaneous or sequential use.

16. Use of a ligand according to any of claims 3 to 11, a humanized or hybridized monoclonal antibody according to claim 12 or an antigen -binding Fab fragment according to claim 13 as a medicament.

17. Use according to claim 16 in simultaneous or sequential association with at least a thrombolytic agent.

18. Use according to claim 16 or claim 17 for the treatment and/or prevention of a disorder of haemostasis.

19. Use according to any of claims 16 to 18, wherein the said medicament is for oral, intranasal, subcutaneous, intramuscular, intradermal, intravenous,

intraarterial or parenteral administration or for catheterization.

20. A polynucleotide encoding for an antigen-binding Fab fragment according to claim 13.

21. A DNA probe for detecting the polynucleotide sequence of claim 20, comprising a nucleic acid molecule having a sequence complementary to the coding sequence of said polynucleotide.

22. A polynucleotide sequence as shown in SEQ.N°1.

23. A polynucleotide sequence as shown in SEQ.N°2.

24. An amino acid sequence as shown in SEQ.N°3.

25. An amino acid sequence as shown in SEQ.N°4.